

Genetic risk for neuroticism predicts emotional health depending on childhood adversity

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Original Article

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Abstract

Background. Existing evidence for gene × environment interaction (G × E) in neuroticism largely relies on candidate gene studies, although neuroticism is highly polygenic. This study aimed to investigate the long-term associations between polygenic risk scores for neuroticism (PRS_N), objective childhood adversity and their interplay on emotional health aspects such as neuroticism itself, depressive symptoms, anxiety symptoms, loneliness and life satisfaction.

Methods. The sample consisted of reared-apart (TRA) and reared-together (TRT) middle- and old age twins ($N = 699$; median age at separation = 2). PRS_N were created under nine p value cut-off thresholds (p_T -s) and the p_T with the highest degree of neuroticism variance explained was chosen for subsequent analyses. Linear regressions were used to assess the associations between PRS_N, childhood adversity (being reared apart) and emotional health. G × E was further investigated using a discordant twin design.

Results. PRS_N explained up to 1.7% ($p_T < 0.01$) of phenotypic neuroticism in the total sample. Analyses across two separation groups revealed substantial heterogeneity in the variance explained by PRS_N; 4.3% was explained in TRT, but almost no effect was observed in TRA. Similarly, PRS_N explained 4% and 1.7% of the variance in depressive symptoms and loneliness, respectively, only in TRT. A significant G × E interaction was identified for depressive symptoms.

Conclusions. By taking advantage of a unique sample of adopted twins, we demonstrated the presence of G × E in neuroticism and emotional health using PRS_N and childhood adversity. Our results may indicate that genome-wide association studies are detecting genetic main effects associated with neuroticism, but not those susceptible to early environmental influences.

Background

Neuroticism is a relatively stable personality trait described by a tendency to experience higher levels of emotional instability, worry and fear. High neuroticism is an established predictor of future mental health problems, especially stress-related disorders such as depression and anxiety (e.g. Kendler *et al.* 2006; Kotov *et al.* 2010). Its influence is also reflected in a broad range of life outcomes, such as lower well-being and higher loneliness in late life (Hensley *et al.* 2012; Mhaolain *et al.* 2012; Kahlbaugh & Huffman, 2017).

Given the temporal stability of neuroticism trait (e.g. Harris *et al.* 2016; Terracciano *et al.* 2017), its development starts already early in life and is likely shaped by genetic predisposition and exposure to childhood adversity (Lahey, 2009; Barlow *et al.* 2014). Animal and human studies have established that early childhood serves as a crucial developmental window in which stress has likely a major influence on persistent stress-related brain functions (Lupien *et al.* 2009; Heim & Binder, 2012) and hence may influence the development of a stress-sensitive personality type. Indeed, individuals exposed to childhood adversity show higher neuroticism levels (Roy, 2002; Rosenman & Rodgers, 2006; Wilson *et al.* 2006) and are at greater risk of adulthood stress-related mental health problems, such as depression and anxiety disorders (Kendler *et al.* 1998; Agid *et al.* 2000; Fava & Kendler, 2000; Heim *et al.* 2004; Shonkoff *et al.* 2012). However, measuring early-life adversity using retrospective approaches, as is currently the common practice, may lack in objectivity and validity. For instance, in case of early occurrence (at age 3 or younger), many stressful events would be difficult to detect retrospectively, mainly due to recall bias, so-called infantile amnesia or just because of not being aware of these problems at very young age (e.g. family's socioeconomic hardship) (Hardt & Rutter, 2004).

Extensive research on heritability of personality has confirmed a substantial genetic component in neuroticism, with heritability estimates averaging around 0.40 (e.g. Polderman *et al.* 2015; e.g. Vukasović & Bratko, 2015). The remaining variance is almost entirely explained by non-shared environment, that in addition to unique experiences and measurement error, can

also encompass gene \times environment interactions ($G \times E$) (Bouchard & Loehlin, 2001). To date, $G \times E$ research in stress-related phenotypes have been largely driven by candidate gene studies, and the results remain controversial (Duncan & Keller, 2011; Border & Keller, 2017). One of the main reasons behind replication difficulties is that the effects of single nucleotide polymorphisms (SNPs) in complex traits, such as neuroticism, are usually very small, thus making it hard to detect reliable evidence for underlying $G \times E$ effects. Neuroticism has a highly polygenic nature and genome-wide association studies (GWAS) have only recently gained enough power to detect significant hits (De Moor *et al.* 2015; Okbay *et al.* 2016; Nagel *et al.* 2017; Luciano *et al.* 2018). Given the contributions of thousands of SNPs explaining the variation in neuroticism, a genetic risk score (polygenic risk score, PRS) approach would be appropriate to simultaneously test the predictive power of many associated SNPs. Moreover, the PRS approach is a substantial advancement in gene-environment interplay research since it provides a useful tool to investigate both the interactions as well as correlations between genes and environments (rGE) (Plomin, 2013; Wray *et al.* 2014).

This study aimed to take advantage of the separated twin design to investigate the effects of genetic risk for neuroticism, objective childhood adversity and their interaction on late-life emotional health aspects such as neuroticism itself, depressive symptoms, anxiety symptoms, loneliness and life satisfaction. Twins being reared apart likely captures a number of stressful early-life adversities, which lead to separation and are eventually reflected in increased risk for mental health problems in adulthood (Melero & Sanchez-Sandoval, 2017). Thus, a sample of reared-apart twins and their matched controls of conventionally reared-together twins allow us to investigate the influence of objective early-life adversity that would be difficult to assess retrospectively in population-based adult samples.

Methods

Sample

The Swedish Adoption/Twin Study of Aging (SATSA) is a longitudinal study in gerontological genetics that followed community-dwelling older twins reared together (TRT) and reared apart (TRA) over a 30-year period (Pedersen *et al.* 1991). The base population is comprised of 351 same-sex MZ and DZ pairs of twins who were separated early in their childhood and reared apart. In addition, the base population also included 407 age, sex, presumed zygosity and county of birth-matched control pairs of twins reared together and additional 502 single twins who responded to a questionnaire in 1984. At the first follow-up, 83 additional individuals responded, resulting in a total of 2101 individuals with any participation. A subsample of twins from full twin pairs underwent thorough in-person testing as well as blood sample donation for genotyping purposes. Thus, genotype information is available for a total of 699 individuals. In this study, we used cross-sectional data from the first assessment occasion of each individual with available genotype data.

Early-life adversity exposure

The SATSA study with its unique adopted twin design enables investigating the effects of being adopted as an objective early-life adversity on various psychological health outcomes decades after

exposure. The median age of separation for TRA was 2 (range = 0–11), and the most prevalent circumstances for separation of twins included illness/death of the mother, the mother being single and/or economic hardship of the household (online Supplementary Fig. S1). TRA have previously shown to have lower socioeconomic status (SES), education levels and weight, but higher neuroticism as adults compared with TRT (Pedersen *et al.* 1984). In addition, TRA also reported lower childhood SES compared with TRT (online Supplementary Fig. S1). Thus, the separation status (reared together or reared apart) is treated as an indicator of serious early-life adversity exposure in this study (coded as: 0 = TRT and 1 = TRA). Although in some cases only one twin was adopted away and/or reared by relatives, e.g. grandmother or aunt, both twins likely experienced pre-adoption risk factors leading to the separation, followed by separation itself. This is also reflected in the additional descriptive information on mean neuroticism and childhood SES levels across groups with different degrees of relatedness to their care takers (online Supplementary Fig. S1).

Outcome measures

Neuroticism

A short form of the Eysenck Personality Questionnaire was used to measure neuroticism (Floderus, 1974). The scale consists of nine items, scored as either yes (1) or no (0) (see Pedersen *et al.* 1988, for more details).

Depressive symptoms

Depressive symptoms were self-reported using the Center of Epidemiological Studies – Depression scale (CES-D), consisting of 20 items, each representing a symptom of depressive disorder (Radloff, 1977). Respondents rate the frequency with which they experienced each symptom during the week prior to completing the questionnaire, from ‘rarely or none of the time’ (0) to ‘most or all of the time’ (3).

Anxiety symptoms

State anxiety was measured by 10 of the 20 items of State-Trait Anxiety Inventory (Spielberger, 1983), scored on a five-point scale (Wetherell *et al.* 2001).

Loneliness

Loneliness was assessed by a single question ‘Are you ever troubled by feelings of loneliness?’. The score range was 1–3 with (1) being ‘quite often’ and (3) ‘hardly ever’, which was reversed for the analysis.

Life satisfaction

SATSA questionnaires included a 13-item Life Satisfaction Index, adapted from (Wood *et al.* 1969), and scored on a five-point response scale from ‘strongly agree’ to ‘strongly disagree’.

Genotyping

Genotypes were generated on the Illumina PsychArray-24 BeadChip, further imputed to 1000 Genome phase 1 version 3 panel. In total, 567 individuals out of 594 passed the QC. Since only one twin of MZ pairs was genotyped, the genotype information was imputed to the co-twin, resulting in total 699 individuals with genotype information.

Genetic risk scores

The genetic risk score approach uses findings from large GWAS, where meta-analysis association results for each SNP investigated in the discovery sample are used to create a genetic risk score for a given phenotype in another, independent sample. This is done by summing risk alleles of each gene variation (0, 1 or 2 alleles) for each SNP, weighting by the effect size derived from the discovery sample, and combining the single weighted values across all loci in the independent sample. The PRS for neuroticism (PRS_N) in this study were created with Plink 1.9 software using previously published summary statistics from a large GWAS, which used pooled Genetics of Personality Consortium (GPC) and UK Biobank data (Okbay *et al.* 2016). As some Swedish twins were included in the original discovery sample, we checked potential overlap with our sample, and identified a total of 62 overlapping individuals and their co-twins. These 62 individuals were omitted from all analyses, yielding a total sample size of 637. In order to deal with linkage disequilibrium (LD), clumping was performed on the discovery association data. This procedure selects most significantly associated SNPs and excludes SNPs in strong LD ($r^2 < 0.1$ within a 1000 kb window using Plink version 1.9). PRS_N were established at nine p value thresholds (p_T) ranging from 5×10^{-8} to 1 and then transformed to z -scores. The numbers of LD-pruned SNPs in each of the nine scores were as follows: $5 \times 10^{-8} = 12$; $1 \times 10^{-5} = 120$; $1 \times 10^{-4} = 463$; $1 \times 10^{-3} = 1910$; $0.01 = 9052$; $0.05 = 27\ 294$; $0.1 = 43\ 214$; $0.5 = 112\ 247$; $1 = 144\ 367$.

Statistical analysis

The phenotypic correlations between the five outcome phenotypes were determined with Spearman's rank correlation coefficients. The presence of gene–environment correlation between genetic risk for neuroticism and being reared apart was investigated by conducting logistic regression analyses separately for each of the nine PRS_N predicting rearing status, adjusted for 5 Principal Components (PC-s) to account for population stratification.

To determine which p value threshold out of the nine PRS_N explains the highest proportion of phenotypic neuroticism in our sample, we first carried out linear regression analyses for each of the nine PRS_N scores predicting neuroticism adjusted for age, age², sex and 5 PC-s, and an additional reduced model with the covariates only. The proportion of variance explained by each PRS_N p_T was determined by comparison of the R^2 in the full (PRS_N and covariates) and reduced (covariates only) model. This procedure was also carried out stratified based on rearing status in order to investigate potential differences in genetic risk prediction across the two separation groups. The p_T with the highest degree of neuroticism variance explained was chosen for all subsequent analyses. A sensitivity analysis was conducted to test the predictive ability and the direction of the association of all nine p_T s also in depressive symptoms, anxiety symptoms, loneliness and life satisfaction. The main effects of PRS_N (model 1) and rearing status (model 2) as well as $G \times E$ interactions (i.e. PRS_N \times rearing status; model 3) were tested using linear regression models for neuroticism, depressive symptoms, anxiety symptoms, loneliness and life satisfaction. All analyses were adjusted for age, age², sex and 5 PC-s. For testing $G \times E$ interactions (model 3), an interaction term for PRS_N \times rearing status was included in the models. In order to adjust for the effects the covariates might have on the interaction effect, we also

included adjustments for all covariate \times PRS_N and covariate \times rearing status interactions (Keller, 2014). To assess the proportion of variance in depressive symptoms, anxiety symptoms, loneliness and life satisfaction explained by PRS_N, R^2 in the full (PRS_N and covariates) and reduced (covariates only) model 1 were compared in the total sample and, for descriptive purposes, additionally stratified based on rearing status. A sensitivity analysis was carried out on main $G \times E$ findings, where additional adjustment for childhood SES was included in the model. Childhood SES used was a composite measure of parental education and occupation as well as family's access to a summer house or a boat.

Since SATSA is entirely composed of related individuals (twins), dependency between observations due to twin design was controlled for by using cluster-robust standard error estimator (i.e. the sandwich estimator) on pair ID in all analyses. Analyses were conducted in Stata/IC 14.0.

Discordant MZ twin design

Since twins can provide informative designs to investigate $G \times E$ effects, a discordant twin design was used as an alternative approach to investigate $G \times E$. We first used a Fisher's test for heterogeneity to test for within-pair difference heterogeneity in MZ twins, which could indicate the presence of $G \times E$ (Reynolds *et al.* 2016). This is an agnostic test to identify the presence of a mixture of distributions, rather than one distribution of the within-pair difference. Next, we tested whether PRS_N contributes to the phenotypic within-pair difference in MZ twins. Within-pair difference scores for all five phenotypes were created by taking the absolute difference of the values of both members of the pair. Linear regression analyses were conducted on main effects as well as with a PRS_N \times rearing status interaction to investigate the influence of PRS_N on the phenotypic differences within MZ twin pairs. Models were adjusted for sex and age. A significant effect would indicate neuroticism's risk alleles acting as variability alleles, meaning their association to trait variation and not just trait mean.

Ethical standards

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All subjects provided informed consent. SATSA study has been reviewed and approved by the Ethical Review Committee at Karolinska Institutet (Ethical review numbers: 84-61, 98-319, 2010/657-31/3).

Results

Sample characteristics and phenotypic correlations

The sample consisted of 310 reared-apart twins (female 58%) and 327 reared-together twins (female 54%). Mean age at testing of neuroticism, anxiety symptoms, loneliness and life satisfaction was 57.1 (s.d. = 11.1). The mean age at measurement of depressive symptoms was 60.8 (s.d. = 11), due to the first measurement of depressive symptoms taking place during a follow-up, 3 years after the baseline assessment. Sample characteristics, phenotypic means as well as phenotypic correlations across two separation groups are shown in Table 1. All five phenotypes under investigation showed moderate-to-strong correlations. Although testing for possible rGE showed a tendency towards separated twins having slightly

Table 1. Phenotypic means and correlations of neuroticism, depressive symptoms, anxiety symptoms, loneliness and life satisfaction across separation groups

					Phenotypic rank correlations across rearing status ^a				
	TRA		TRT		TRA\TRT				
	N	Mean (s.d.)	N	Mean (s.d.)	Neur	DS	AS	Lonely	LS
Age	310	57.31 (11.11)	327	58.41 (11.00)					
Neuroticism (Neur)	287	2.99 (2.42)	315	2.49 (2.22)	1	0.37	0.30	0.34	-0.37
Depressive symptoms (DS)	269	10.50 (8.30)	291	10.09 (8.06)	0.37	1	0.44	0.33	-0.37
Anxiety symptoms (AS)	255	19.50 (8.63)	276	17.92 (7.01)	0.44	0.44	1	0.34	-0.48
Loneliness (Lonely)	265	1.58 (0.70)	296	1.53 (0.66)	0.30	0.42	0.42	1	-0.41
Life satisfaction (LS)	280	45.26 (8.60)	307	47.73 (7.32)	-0.41	-0.40	-0.42	-0.40	1

TRA, twins reared apart; TRT, twins reared together; s.d., standard deviation.

^aAll correlations are significant.

higher genetic risk for neuroticism than non-separated twins under more stringent p_T -s, only $PRS_N p_T < 1 \times 10^{-4}$ was a significant predictor of being reared apart (online Supplementary Table S1).

Neuroticism’s variance explained by common SNPs

Out of the nine $PRS_N p_T$ -s investigated, $PRS p_T < 0.01$ explained the highest proportion of variance in neuroticism (1.7%) in the total sample (Fig. 1). Separate analyses in TRA and TRT groups revealed significant heterogeneity in the variance explained by PRS_N , where the effects of PRS_N were significantly attenuated in the reared-apart group. $PRS_N p_T < 0.01$ appears to stand out in the reared-together group, explaining 4.3% of the variance in neuroticism, and thus we continued with $PRS_N p_T < 0.01$ in our next analyses.

Effects of PRS_N , rearing status and their interaction on neuroticism, depressive symptoms, anxiety symptoms, loneliness and life satisfaction

$PRS_N p_T < 0.01$ did not significantly predict depressive symptoms, anxiety symptoms, loneliness or life satisfaction in the total

sample (Table 2, model 1). Sensitivity analyses for the main effects of all $PRS_N p_T$ -s can be found in the online Supplementary Table S2. Being separated in early childhood significantly predicted higher neuroticism and anxiety symptoms, as well as lower life satisfaction (Table 2, model 2). Model 3 revealed large point estimates for the interaction between the PRS_N and rearing status on depressive symptoms and approached significance for neuroticism and loneliness (Table 2, model 3). An additional sensitivity analysis was conducted to examine whether childhood SES could have influenced the $G \times E$ for depressive symptoms, but the interaction term remained significant after the adjustment ($B = -2.22$; $p < 0.005$).

Analysis stratified by rearing status revealed that higher $PRS_N p_T < 0.01$ predicted higher depression scores in TRT ($B = 1.73$; 95% CI 0.55–2.90; $p < 0.005$), but not in TRA ($B = -0.44$; 95% CI -1.36 to 0.46; $p = 0.3$) (Fig. 2). $PRS_N p_T < 0.01$ explained considerable amount of variance in depressive symptoms (4.0%) and in loneliness (1.7%) only in the TRT group (Fig. 3).

Discordant MZ twin design

For the discordant MZ twin design, we identified 103–127 MZ pairs with available data on the five phenotypes under investigation. Fisher’s test of heterogeneity revealed mixture distributions for neuroticism ($p < 0.0001$), depressive symptoms ($p < 0.018$) and life satisfaction ($p < 0.022$) (loneliness was excluded because the test assumes interval-level data). The within-pair difference analysis suggested that the $PRS_N p_T < 0.01$ may be a significant predictor of depressive symptom within-pair difference only ($p = 0.03$), although the model was not significant ($p = 0.07$) (online Supplementary Table S3). We therefore ran an extra likelihood ratio test to check whether PRS_N makes a significant improvement to the model fit and found that PRS_N significantly improves the model. We therefore concluded that PRS_N is a significant predictor of within-pair differences in depressive symptoms. The $PRS_N \times$ rearing status interaction was not significant for any of the five phenotypes.

Discussion

By using a unique adopted-twin sample, we conducted a gene–environment interplay study on the association of neuroticism’s genetic risk and early separation of twin pairs on neuroticism, depressive symptoms, anxiety, loneliness and life satisfaction in

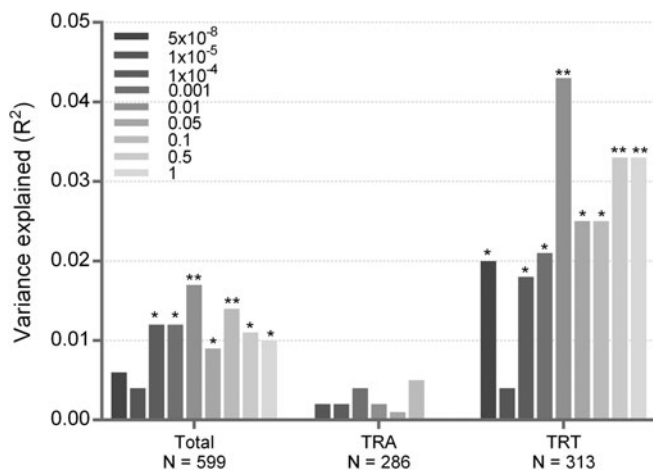


Fig. 1. Neuroticism variance explained by neuroticism-associated common SNPs at nine p value thresholds in total sample ($N = 599$), twins reared apart ($N = 286$) and twins reared together ($N = 313$). Notes: Variance refers to ΔR^2 between full and reduced regression models. * $p < 0.05$; ** $p < 0.01$.

Table 2. Main and interaction effects of PRS_N and rearing status on neuroticism, depressive symptoms, anxiety symptoms loneliness and life satisfaction in middle- and late life

	Main effect of PRS _N (model 1) ^a			Main effect of rearing status (model 2) ^b			PRS _N × rearing status (model 3) ^c		
	<i>B</i>	95% CI	<i>p</i>	<i>B</i>	95% CI	<i>p</i>	<i>B</i>	95% CI	<i>p</i>
Neuroticism	0.31	0.11 to 0.51	<0.01	0.47	0.04 to 0.89	0.03	-0.36	-0.76 to 0.05	0.08
Depressive symptoms	0.68	-0.08 to 1.44	0.08	0.28	-1.15 to 1.72	0.70	-2.18	-3.63 to -0.72	<0.01
Anxiety symptoms	0.26	-0.51 to 1.02	0.51	1.50	0.07 to 2.92	0.04	-0.56	-2.18 to 1.05	0.49
Loneliness	0.03	-0.03 to 0.10	0.29	0.04	-0.07 to 0.15	0.49	-0.11	-0.23 to 0.02	0.09
Life satisfaction	-0.29	-0.99 to 0.41	0.42	-2.43	-3.9 to -0.96	<0.01	0.29	-1.13 to 1.72	0.69

^aModels included the main effect of PRS_N $p_T < 0.01$ and adjustments for age, age², sex and 5 PC-s.

^bModels included rearing status as main effect and adjustments for age, age² and sex.

^cModels included PRS_N and rearing status main and interaction effects, further adjusted for age, age², sex, 5 PC-s, and for all PRS_N × covariate and rearing status × covariate interactions; *B*, unstandardized regression coefficient indicating change per one s.d. increase in PRS_N (model 1) or being reared apart (model 2), or the change in the coefficients for TRT v. TRA (model 3); CI, confidence intervals.

middle- and old age. In the total sample, PRS_N predicted only phenotypic neuroticism, and being reared apart was associated with higher neuroticism, having more anxiety symptoms and lower life satisfaction. Further G × E analyses revealed considerably stronger effect of PRS_N on neuroticism in the reared-together twins, suggesting heterogeneity in neuroticism development depending on childhood adversity. The interaction between PRS_N and rearing status was significant for depressive symptoms and showed a similar pattern for neuroticism and loneliness.

Early childhood is considered as a developmental period with high importance in terms of brain development. Adverse experiences in early life may alter neuronal pathways in the central nervous system probably through stable epigenetic modifications, leading to higher susceptibility to stress-related health problems (Heim & Binder, 2012). Compared with children raised by their biological families, adopted children are at higher risk for psychological adjustment problems, including higher levels of depression and anxiety (Sharma *et al.* 1996; Juffer & van IJzendoorn, 2005; Melero & Sanchez-Sandoval, 2017), possibly reflecting the increased exposure to pre-adoptive risk factors, such as abuse, neglect or losing a care taker. Previous studies exploiting natural

experiments with individuals who were separated from their families as young children during WWII found altered hypothalamic-pituitary-adrenal axis responses (Pesonen *et al.* 2010) and more severe depressive symptoms (Pesonen *et al.* 2007) compared with non-separated individuals. We did find higher neuroticism, more anxiety symptoms and loneliness in reared-apart twins, but no significant differences in depressive symptoms emerged, despite early-life adversity being an established risk factor for depression. Late-life depressive symptoms are likely associated with ageing-related neurobiological changes in the brain, cognitive diathesis and recent stressful events, rather than early-life adversity (Fiske *et al.* 2009). Early-life adversity and late-life depression associations have been previously investigated primarily using retrospective adversity measurements; however, this approach may induce a substantial recall bias in older individuals when asked about very distant childhood events/circumstances (Gershon *et al.* 2013). Therefore, focusing on more objective measures of early-life adversity may greatly contribute to the understanding of early adversity's role in late-life depression.

Although neuroticism has been found to predict stressful life events (e.g. Magnus *et al.* 1993; Kendler *et al.* 2003), gene-environment correlation studies investigating common genetic

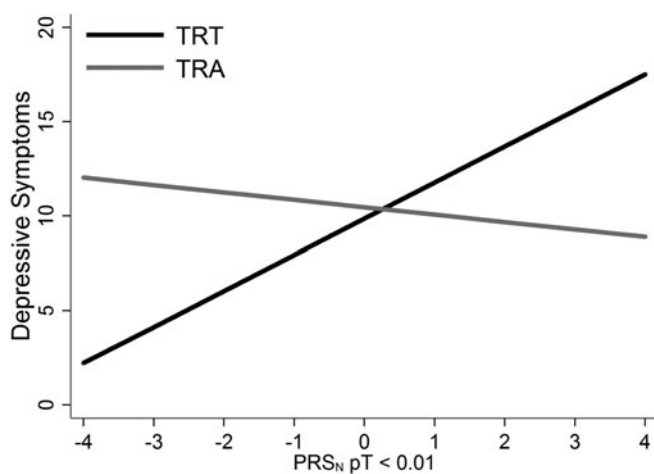


Fig. 2. Linear prediction of the interaction between genetic risk for neuroticism and rearing status on depressive symptoms in middle- and old age. TRT, twins reared together; TRA, twins reared apart.

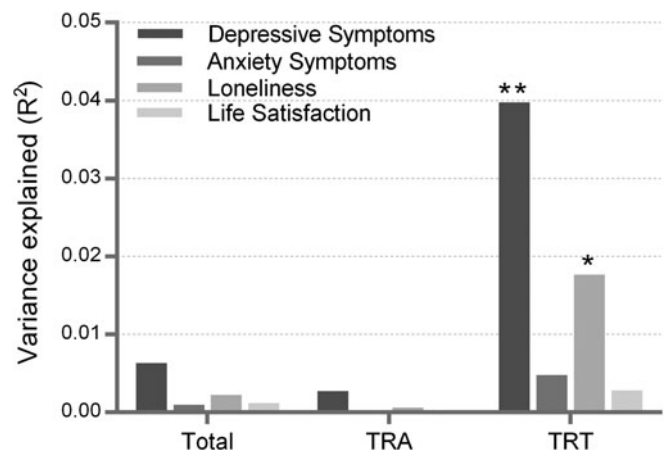


Fig. 3. PRS $p_T < 0.01$ explaining variance in depressive symptoms, anxiety symptoms, loneliness and life satisfaction. TRA, twins reared apart; TRT, twins reared together; * $p < 0.05$; ** $p < 0.01$.

base in neuroticism and life stress exposure have led to inconsistent findings (Saudino *et al.* 1997; Os *et al.* 2001; Middeldorp *et al.* 2008). To our knowledge, this is the first study addressing rGE in the neuroticism–early-life stress association using PRS. Although we found slightly higher genetic risk for neuroticism in reared-apart twins, out of nine p value cut-offs used, only one was a significant predictor of being reared apart and would likely not survive more stringent correction for multiple testing (over nine tests). Furthermore, this specific p_T was not the strongest predictor of phenotypic neuroticism in our sample. Thus, based on our results, there was no strong evidence to support the presence of true gene–environment correlation, i.e. TRA having higher genetic predisposition for neuroticism compared with TRT. However, future studies on larger samples are needed to address the possible rGE using PRS_N.

PRS_N predicted phenotypic neuroticism in reared-together twins, but not in reared-apart twins. Such a pattern is counter-intuitive in the context of the well-known diathesis – stress model proposed to explain the aetiology of stress-related psychiatric problems (e.g. Monroe & Simons, 1991). Since childhood adversity has been established as a significant determinant of higher neuroticism and related traits, and our data support this, such early experiences might have stronger impact on neuroticism development in comparison to common SNPs that have been detected by large GWAS. As another possible explanation, these findings could be driven by the specific characteristics of the GWAS discovery sample. The PRS_N were derived from a large GWAS meta-analysis conducted mainly on population-based samples where the environmental adversity was not accounted for and the majority of participants had likely not been exposed to serious early-life stress. Thus, this may explain why the PRS predicts neuroticism and related emotional health only in reared-together, but not in reared-apart twins in this sample. Interestingly, although our MZ within-pair difference analysis detected the presence of a mixture of distributions in the variation of neuroticism, depressive symptoms and life satisfaction, we did not detect significant interaction effect between PRS_N and rearing status on trait variation, probably because of being underpowered with just 103–127 available pairs. However, these common SNPs may act as variability genotypes influencing depressive symptom difference. All this suggests that the GWAS results may be tapping into the genetic main effects associated with neuroticism or genetic effects interacting with other environments, but probably not those that are susceptible to early environmental influences. This is warranting future efforts in conducting gene-by-environment GWAS in order to identify genetic effects more strongly susceptible to environments.

While applying genetic risk scores in $G \times E$ studies is still a rather new approach, no such studies are currently available on $G \times E$ in neuroticism. Nevertheless, three reports can be found on major depressive disorder (MDD) (Peyrot *et al.* 2014; Mullins *et al.* 2016) and depression symptoms (Musliner *et al.* 2015). Mullins *et al.* and Peyrot *et al.* both focused on MDD PRS and found significant interactions with self-reported childhood trauma, but the results were not consistent. While Peyrot *et al.* found that the effect of PRS on MDD is increased in the presence of childhood trauma, Mullins *et al.* showed a similar trend in individuals without childhood trauma but the opposite association in moderate/severe childhood trauma group. Our results, using PRS for neuroticism, are consistent with the latter findings, since we found PRS_N to predict neuroticism, depressive symptoms and loneliness more strongly in the reared-together

group, e.g. individuals without exposure to adoption-related stress in early childhood. Although our focus was on genetic risk for neuroticism and not MDD, neuroticism and depression share a notable amount of genetic variance (e.g. Kendler *et al.* 2006; Kendler & Myers, 2010; Lo *et al.* 2017), which may explain why we see similar patterns using PRS_N. Furthermore, our findings are generally in line with previous reports showing PRS_N predicts depression (Middeldorp *et al.* 2011; De Moor *et al.* 2015), although the association is observed only in reared-together twins in our sample. Interestingly, despite the strong genetic correlation between neuroticism and anxiety, the PRS_N did not predict anxiety symptoms in our sample. The results of the sensitivity analysis suggest that other p value thresholds could be better predictors of anxiety symptoms and that more powerful discovery GWA studies would likely improve the predictive accuracy of PRS_N.

Although loneliness and life satisfaction have not received comparable attention as depression, a few relevant reports, also focusing on genetic risk prediction, have been published lately. In a recent report, Gao *et al.* highlight a significant genetic correlation between loneliness and neuroticism as well as an association between PRS_N and loneliness (Gao *et al.* 2017), which our results support. One source for possible overlap may stem from the sense of negative effect both phenotypes refer to. Alternatively, this association could be explained by the partial genetic and phenotypic overlap between neuroticism and depression, and that the measurement instrument applied for the assessment of depression also includes items tapping into loneliness (e.g. 'I felt lonely'). Regarding life satisfaction, another recent report has focused on investigating the effect of PRS_N on life satisfaction and positive affect, revealing significant impact on life satisfaction (Weiss *et al.* 2016). We did not replicate this finding, which may be due to differences in the GWAS summary statistics used, the assessment method of life satisfaction or our significantly smaller sample of older individuals.

This study has several strengths. The unique sample design allows investigating the effects of objective early-life adversity on eventual neuroticism and related psychological phenotypes decades after exposure. Vast majority of studies investigating early adversity rely on retrospective assessments, which may confer major biases. Furthermore, a polygenic risk estimation approach was used in this study, which is a substantial advancement beyond candidate genes to investigate gene–environment interplay in stress-related phenotypes. Nevertheless, despite using a powerful discovery GWAS, the increasing sample sizes of upcoming GWASs are providing hope for improved accuracy of genetic risk estimation in the future, thus warranting replication attempts using more powerful predictors. In addition, accumulating evidence about gene–environment interplay in neuroticism and related phenotypes emphasizes the need for large genome-wide by environment interaction studies to investigate heterogeneity in SNP effects that may depend on life adversity.

However, some weaknesses of this study need addressing. Our sample consisted of a relatively small number of middle-aged and older adults, which highlights the need for future replication attempts on larger and different age range samples before major conclusions can be drawn. Despite being an objective measure of early adversity, rearing status as such does not provide us with information on specific environmental sources of adversity, life events leading to separation or the stress levels experienced. Further longitudinal studies are needed to ascertain the role of

specific environmental stressors in order to pave the way for better prediction of high-risk individuals and improved intervention strategies.

To conclude, by using PRS in combination with childhood adversity, we demonstrated the presence of $G \times E$ in middle- and late-life neuroticism and depressive symptoms. Further studies are needed to disentangle the genetics underlying stress-related psychiatric phenotypes and their aetiological heterogeneity depending on early-life adversity. With improved predictive accuracy provided by more powerful GWAS and understanding the role of stress exposure, we can move towards better prevention and intervention strategies of stress-related disorders in the future.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0033291718000715>.

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